

Article

The Effects of Land-Use Change from Grassland to *Miscanthus x giganteus* on Soil N₂O Emissions

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Abstract: A one year field trial was carried out on three adjacent unfertilised plots; an 18 year old grassland, a 14 year old established *Miscanthus* crop, and a 7 month old newly planted *Miscanthus* crop. Measurements of N₂O, soil temperature, water filled pore space (WFPS), and inorganic nitrogen concentrations, were made every one to two weeks. Soil temperature, WFPS and NO₃⁻ and NH₄⁺ concentrations were all found to be significantly affected by land use. Temporal crop effects were also observed in soil inorganic nitrogen dynamics, due in part to C₄ litter incorporation into the soil under *Miscanthus*. Nonetheless, soil N₂O fluxes were not significantly affected by land use. Cumulative yearly N₂O fluxes were relatively low, 216 ± 163, 613 ± 294, and 377 ± 132 g·N·ha⁻¹·yr⁻¹ from the grassland, newly planted *Miscanthus*, and established *Miscanthus* plots respectively, and fell within the range commonly observed for unfertilised grasslands dominated by perennial ryegrass (*Lolium perenne*). Higher mean cumulative fluxes were measured in the newly planted *Miscanthus*, which may be linked to a possible unobserved increase immediately after establishment. However, these differences were not statistically significant. Based on the results of this experiment, land-use change from grassland to *Miscanthus* will have a neutral impact on medium to long-term N₂O emissions.

Keywords: land-use change; N₂O; bioenergy crops; *Miscanthus*

1. Introduction

The agriculture and energy sectors are major contributors to greenhouse gas emissions, globally accounting for 13.5% and 25.9% of all anthropogenic emissions respectively [1]. In 2009, Irish agriculture was responsible for 29.1% of all national anthropogenic greenhouse gas emissions, the highest proportion of any sector, followed by energy at 21.1% [2]. In Western Europe, soil N₂O emissions are the dominant source of agricultural greenhouse gas emissions [3]. In Ireland, N₂O accounts for 38% of all agricultural greenhouse gas emissions, the vast majority of which is produced in the soil [4].

A potential mechanism to reduce greenhouse gas emissions from both these sectors is to use existing agricultural land for bioenergy crop production with perennial crops. In Ireland, 61% of the land cover is used for agriculture; of this 79% is dedicated to pasture, hay and grass silage, 11% to grazing and 10% to crop production [5]. Articles 3.3 and 3.4 of the Kyoto Protocol encourage the sequestration of atmospheric CO₂ through changes in land use and management as a way to offset CO₂ emissions, and bioenergy crops have been identified to show the greatest potential for carbon mitigation of all the available agricultural options [6]. Ireland has a significant potential for land-use change to bioenergy crops due to the large availability of agricultural land. The Irish government has highlighted the contribution non-food crops can make to reducing greenhouse gas emissions, specifically discussing *Miscanthus* as part of a national strategy for carbon mitigation [7]. The need for alternate farming practices has also been emphasised by the decoupling of EU direct payments from agricultural production, to land-area-based payments, hastening a trend for decreasing livestock numbers in Ireland [8].

As crops grow they fix CO₂ from the atmosphere, this CO₂ being released when the crops undergo combustion implying that the net atmospheric CO₂ levels remain unchanged. This has led to the use of the term “Carbon neutral” in reference to energy produced from biomass [9]. Yet in reality this is a misrepresentation. There is a net CO₂ output associated with energy crops due to the external energy costs associated with the planting, fertilisation, harvesting, and processing of the crops [10]. Other greenhouse gas costs—importantly N₂O emissions—are often overlooked or crudely calculated when analysing the environmental impacts of bioenergy crops. Crutzen *et al.* [11] have indicated that for many first generation bioenergy crops such as rapeseed and maize the negative impacts of N₂O emissions on radiative forcing outweigh the benefits achieved by the subsequent fossil fuel savings, but the same study did suggest that these results may be more favourable for grasses and woody coppices. One such grass gaining popularity in northern Europe as an energy crop is *Miscanthus x giganteus* [8].

Miscanthus x giganteus is a C₄ grass originating from Asia, which has shown encouraging results when grown as a bioenergy crop in temperate climates. Field trials have produced yields of 10–30 t·ha⁻¹·yr⁻¹ across Europe [12], and 13–14 t·ha⁻¹·yr⁻¹ in Ireland at delayed spring harvest [12,13]. *Miscanthus* is a perennial rhizomatous crop with limited fertiliser requirements [14], and planting required roughly only every 15 years [9]. These attributes are favourable for bioenergy crops as they significantly reduce the external energy costs associated with their life cycle. A land-use change from conventional agricultural crops to *Miscanthus* may also lead to an increase in the quantity and quality of plant litter. Plant litter can account for up to almost 20% of total *Miscanthus* above ground

biomass [15], when combined with large yields there is potential for a large quantity of litter to become incorporated into the soil. The quality of this litter is likely to be affected by the fact that C₄ crops tend to contain larger proportions of recalcitrant tissues than C₃ species [16,17]. These changes affect below ground substrate availability and consequent microbial community dynamics [18,19], in turn influencing the soil nitrogen balance and impacting processes such as nitrification, denitrification, and therefore N₂O emissions. More immediate effects on soil N₂O production are also likely following the establishment of *Miscanthus*. Recent research has found that land use change from grassland to another low input second generation bioenergy cropping system, short rotation woody crops (SRWC), can lead to significant short-term increases in N₂O emissions, due to an acceleration of soil carbon and nitrogen cycling associated with the clearing and cultivation of grasslands [20,21].

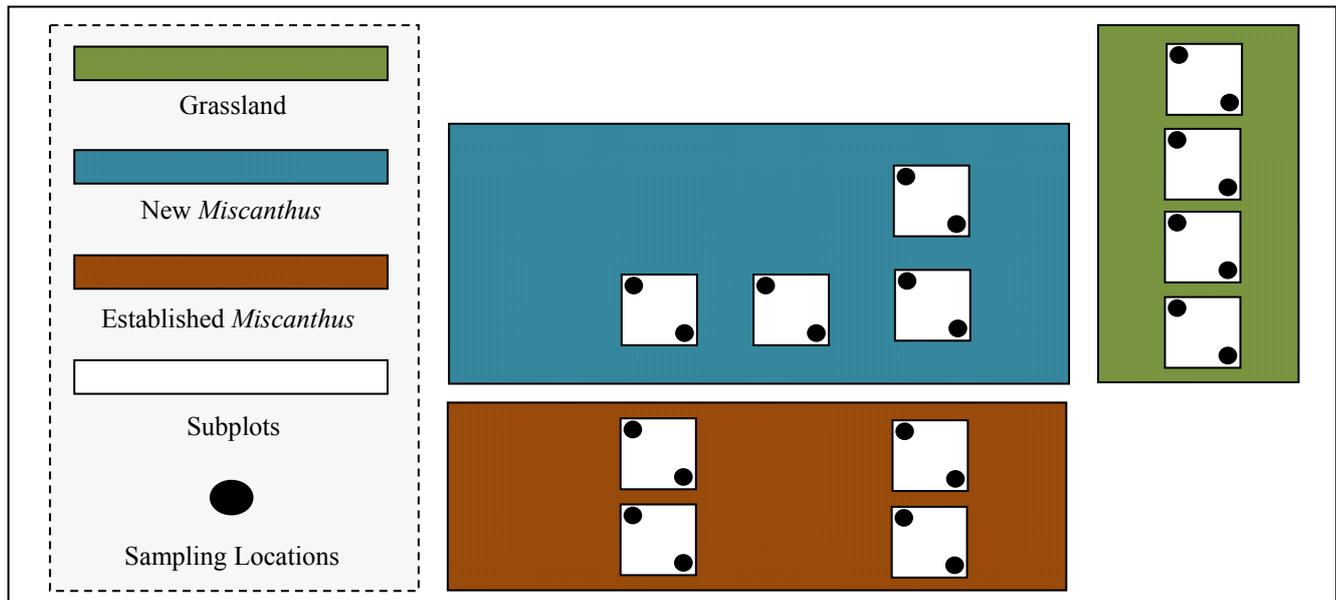
A better understanding of the environmental impacts of land-use change to bioenergy crops is clearly required. This research has focused on the effects of land-use change from an unfertilised, un-grazed grassland (*Lolium perenne*) to *Miscanthus x giganteus*, on soil N₂O fluxes. Here we address the question—does land-use change from *Lolium perenne* to *Miscanthus x giganteus* lead to a significant change in soil N₂O emissions?

2. Materials and Methods

2.1. Site Description

The research presented here was carried out at Teagasc, Crop Research Centre, Oak Park, Co. Carlow, Ireland (52°51'N, 6°54'W, 50 m a.s.l.). The soil is classified as loamy sand with a pH of 6.8 and a mean C/N ratio of 10.7 [22]. The site has a mean annual precipitation of 830 mm and mean temperature of 9.3 °C for the period 1982–2002. During the study period there was rainfall of 946 mm and a mean temperature of 9.4 °C, with minimum and maximum temperatures of −7.0 °C to 26.8 °C. Meteorological data was collected from a station within 1 km of the site (Climate records supplied by Met Eireann, Irish Meteorological Service).

Measurements were carried out over a one year period from November 2008 to November 2009, in three adjacent plots; an 18 year old *Lolium perenne* grassland (approx. 0.3 ha), a 14 year old established *Miscanthus* crop (approx. 0.3 ha), and a seven month old newly planted *Miscanthus* crop (approx. 0.6 ha), herein referred to as G, EM, and NM respectively. All three received minimum management, with no fertiliser applied to any since 2002. The grassland was cut bi-annually in May and September, while the *Miscanthus* was harvested in April. A mean dry matter yield of 16 t·ha⁻¹ was measured at delayed harvest from the established *Miscanthus* in April 2008. The grassland and established *Miscanthus* were converted from a former arable site in 1990 and 1994 respectively, while the newly planted *Miscanthus* was converted from the grassland in 2008. Here, grassland was sprayed with herbicide (Roundup, Monsanto) once in February and again in April before being conventionally tilled and planted with *Miscanthus* at a density of two rhizomes per m² in May. The plots were arranged in a split-plot design with land-use as the main treatment. Each consisted of four subplots (16 m²) from which two samples were taken from opposing corners, resulting in a zigzag sampling pattern (Figure 1).

Figure 1. Diagram of experimental layout (not to scale).

2.2. N_2O Emissions

N_2O emissions were measured on 44 occasions at either one or two week intervals between November 2008 and November 2009 using non-flow-through, non-steady-state chambers (surface area: 0.0314 m^2 , height: 0.194 m , volume: 5 L). Two chambers at opposite ends of each subplot were attached to collars positioned permanently in the soil at a depth of 0.1 m —meaning a total of eight chambers per plot. 22 mL gas samples were taken using a 60 mL syringe and stored in an evacuated glass vial with a butyl rubber/PTFE septum. Samples were taken at 0.5 min after chamber deployment to allow for equilibration inside the chamber, and again after 40.5 min . Measurements were starting at 10.30 , as morning has been found to give the best approximation of daily average flux [23]. Linearity was tested from four random chambers over five points at the start of the experiment (mean $R^2 = 0.94$), and then again over three points in February 2009 (mean $R^2 = 0.90$), May 2009 (mean $R^2 = 0.87$), and August 2009 (mean $R^2 = 0.90$). Samples were analysed for N_2O within one month of collection using a gas chromatograph fitted with an electron capture detector (Clarus 500 GC, Perkin-Elmer, Waltham, MA, USA) and calibrated using standard concentrations of $0, 0.2, 2$ and $10 \text{ ppm } N_2O$. N_2O fluxes are calculated using the following formula derived from Hutchinson and Livingston [24]:

$$F = \left(\frac{\partial C}{\partial t}\right) \left(\frac{M_m}{V_m}\right) \left(\frac{V}{A}\right) \quad (1)$$

where C is the N_2O concentration in the chamber (ppm), t is chamber deployment time, M_m is the molar mass of N_2O (g), V_m is the molar volume of N_2O (m^3), t is time (hours), V is the chamber volume (m^3) and A is the surface area covered by the chamber (m^2). Cumulative annual emissions were calculated by plotting N_2O flux against time, interpolating linearly between the points and then integrating beneath the line [25].

2.3. Ancillary Measurements

Replicated measurements of soil temperature and volumetric water content ($n = 4$) were made alongside N_2O measurements, using a WET sensor (Delta-T). Measurements were made at a depth of 0–10 cm in a 0.25 m^2 area surrounding the soil collars. Mean bulk density for each treatment (0–10 cm) was measured once in March 2009 using soil cores. Volumetric water content was converted into water-filled pore space (WFPS) using measured soil bulk density and a particle density of $2.65 \text{ g}\cdot\text{cm}^{-3}$ [26]. Soil samples were collected monthly from a depth of 0–10 cm for analysis of nitrate and ammonium concentrations ($n = 8$). Samples were collected from a 1 m^2 area surrounding the soil collars. Nitrate and ammonium were extracted from the soil samples based on the techniques described in Compton and Boone [27]. Samples were homogenized and manually sieved through a 2 mm mesh. A 10 g subsample was taken from each sample. This was then added to 100 mL of 2 Molar KCl and shaken for one hour at 135 rpm. The resulting extract was passed through filter paper ($<2.5 \mu\text{m}$), and the filtrate frozen until ready for analysis using a colorimeter (QuickChem QC8500 Automated Ion Analyzer, Lachat, Milwaukee, WI, USA).

2.4. Statistical Analysis

The experiment was analysed as a split-plot design with land use as the main treatment. After testing for normal Gaussian distribution a constant was added to all N_2O flux data, which were then log transformed, while an inverse transformation was applied to soil NH_4^+ concentration. A repeated measures two-way ANOVA followed by a Bonferroni post-test was used in order to test the effects of plot and time on N_2O flux, WFPS, soil temperature, nitrate and ammonium concentrations (Prism 5, Graph Pad). Multiple regression analyses were also carried out for N_2O flux versus these variables and their interactions, using stepwise bidirectional elimination to determine the most suitable models (R 3.0.1). The resulting models were then tested for autocorrelation with a Durbin-Watson test (R 3.0.1). A one-way ANOVA followed by a Tukey's multiple comparison post-test was performed on bulk density data, and yearly cumulative N_2O emissions (Prism 5, Graph Pad). All data were expressed as mean \pm standard error (SE) values.

3. Results

3.1. Nitrous Oxide Flux

Figure 2 shows mean N_2O flux (\pm SE, $n = 8$) from all land uses from November 2008 to November 2009. Mean fluxes showed large variation within treatment, with G ranging from -75 to $37 \mu\text{g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{hr}^{-1}$, NM ranging from -59 to $70 \mu\text{g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{hr}^{-1}$ and EM ranging from -43 to $76 \mu\text{g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{hr}^{-1}$. The majority of the positive and negative peaks occurred between the dates 22 April 2009 and 22 July 2009. Analysing these data by repeated measures two-way ANOVA showed that time ($p = 0.0109$, $df = 43$) and interaction ($p = 0.0129$, $df = 86$) had a significant effect on N_2O flux, but land use did not ($p = 0.5293$, $df = 2$). It should also be noted that N_2O consumption was frequent, with uptake observed in 36%, 40% and 42% of all measurements in NM, EM and G respectively.

Figure 2. Mean N₂O Flux ($\mu\text{g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{hr}^{-1}$) \pm SE (n = 8) from three land uses; G (●), NM (■) and EM (▲). Lines indicate the dates 22 April 2009 and 22 July 2009.

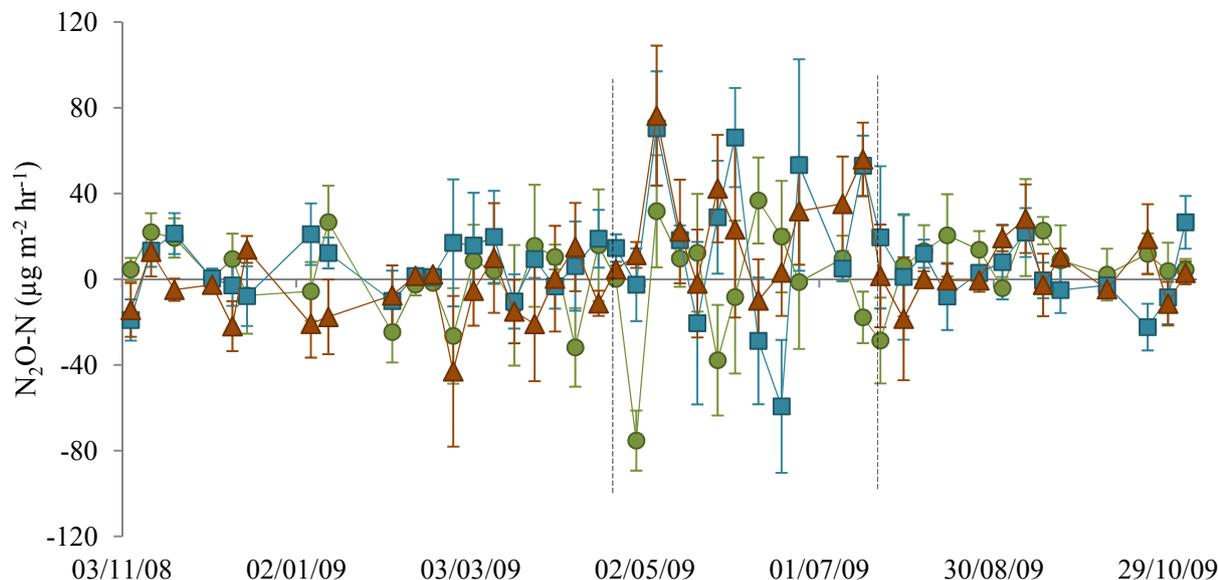


Figure 3 illustrates cumulative N₂O fluxes from each plot over the course of the year. Fluxes were highest from the newly planted *Miscanthus* ($614 \pm 294 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$), as was the case for the majority of the year. Cumulative fluxes from the established *Miscanthus* were $378 \pm 133 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$. This was influenced by a period of consistent uptake between December 2008 and April 2009, and then a period of relatively high emissions between April and August 2009. The lowest mean yearly cumulative flux was seen in the grassland ($217 \pm 164 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$). A one-way ANOVA of these data showed that land use had no significant effect. A subsequent Tukey’s multiple comparison post-test also showed no significant difference in yearly cumulative N₂O flux between any of the treatments. Best fit multiple regression analyses were carried out to test which factors control N₂O flux in each of the treatments. WFPS, soil temperature, soil nitrate and ammonium concentrations, and their interactions were examined. The resulting models were as follows:

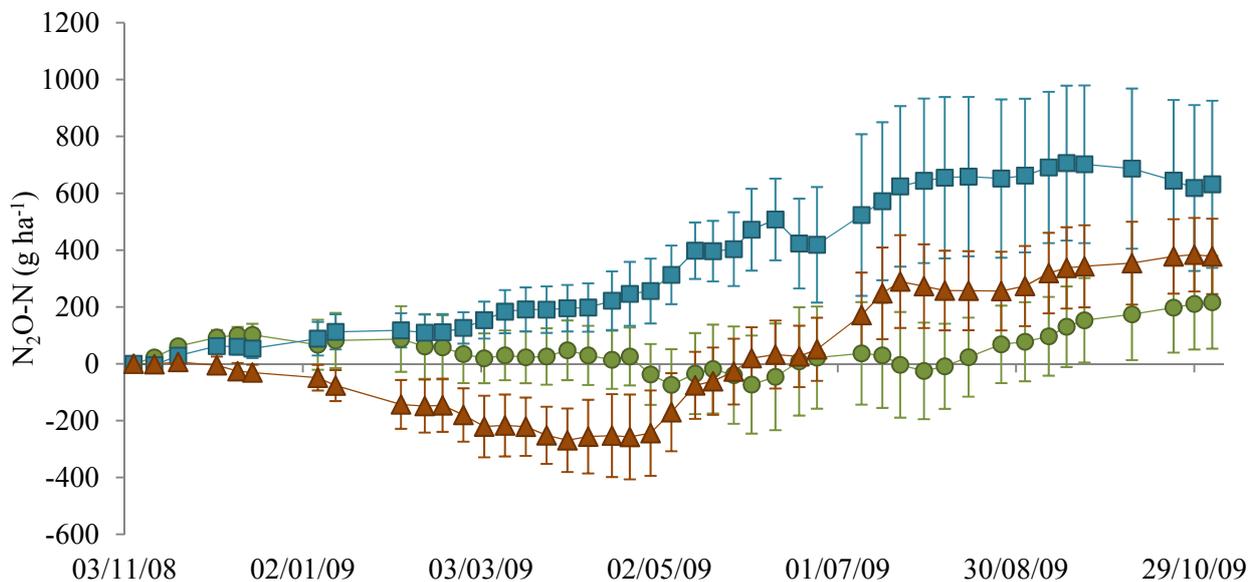
$$G: N_2O = NH_4 \tag{2}$$

$$NM: N_2O = NO_3 + NH_4 + WFPS + Temp + (NO_3)(NH_4) + (NO_3)(WFPS) + (NO_3)(Temp) + (NH_4)(WFPS) + (NH_4)(Temp) + (WFPS)(Temp) + (NO_3)(NH_4)(Temp) + (NO_3)(NH_4)(WFPS)(Temp) \tag{3}$$

$$EM: N_2O = WFPS + Temp + (NO_3)(NH_4) + (WFPS)(Temp) + (NO_3)(NH_4)(WFPS) + (NO_3)(NH_4)(Temp) + (NO_3)(NH_4)(WFPS)(Temp) \tag{4}$$

Results were inconsistent, with N₂O flux poorly explained across the board. 27% of N₂O fluxes from NM were found to be controlled by a large array of factors and complex interactions. Of these, the interaction between soil NO₃⁻ concentration, soil NH₄⁺ concentration, WFPS, and soil temperature was found to be the most significant ($p = 0.0002$). Meanwhile, G and EM responded very poorly with only 1% and 9% of N₂O fluxes accounted for by the regression analyses respectively. No significant relationship was found between any factor or interaction and N₂O, from either land use. Analyses by Durbin-Watson tests indicated that the models were not affected by autocorrelation.

Figure 3. Cumulative N₂O Flux (g·N·ha⁻¹) ± SE (n = 8) over one year from three land uses; G (●), NM (■) and EM (▲).



3.2. Soil Bulk Density, WFPS, and Soil Temperature

Mean soil bulk densities were 0.76 ± 0.02 , 0.94 ± 0.04 , and 0.87 ± 0.05 g·cm⁻³ in G, NM and EM respectively. Land use was shown to have a significant effect on bulk density by a one-way ANOVA ($p = 0.0086$). Specifically a significant difference was seen between G and NM, but not between G and EM, or NM and EM. Soil moisture (0–10 cm) was measured on each sampling date. WFPS was calculated using these measurements and bulk density. Figure 4a shows mean WFPS and standard error (n = 8) in each plot measured from November 2008 to November 2009. Analysis by repeated measures two-way ANOVA showed land use ($p < 0.0001$, $df = 2$), time ($p < 0.0001$, $df = 11$), and interaction ($p < 0.0001$, $df = 22$) to all have a significant effect on WFPS. Further analysis found land use to lead to a significant difference in WFPS between G and EM (19 dates), and NM and EM (22 dates) on a much greater number of occasions than between G and NM (3 dates). In general higher values were observed in EM than the other two. Figure 4b shows mean soil temperature and standard error (n = 8) from each land use measured from November 2008 to November 2009. Mean soil temperature ranged from 26.5 °C in plot NM to -4.7 °C in G. This low temperature was an exception with mean soil temperatures consistently found to be less in EM. Analysis by repeated measures two-way ANOVA showed land use ($p < 0.0001$, $df = 2$), time ($p < 0.0001$, $df = 11$), and interaction ($p < 0.0001$, $df = 22$) to all have a significant effect on soil temperature. Further analysis by a Bonferroni post-test showed land use to lead to a significant difference in soil temperature between all treatments, with significant differences found on a large number of occasions.

3.3. Soil Nitrate and Ammonium

Figure 5 illustrates soil NO₃⁻ (Figure 5a) and NH₄⁺ (Figure 5b) concentrations from all three treatments. Soil NO₃⁻ (0–10 cm) was measured monthly (n = 8). G frequently contained the lowest mean concentrations, the least being 0.9 kg·ha⁻¹ measured on 24 June 2009. Both NM and EM tended

to contain higher concentrations of NO_3^- , with the highest ($11.5 \text{ kg} \cdot \text{ha}^{-1}$) being measured in NM on 24 June 2009. Analysis by repeated measure two-way ANOVA showed land use ($p < 0.0001$, $df = 2$), time ($p < 0.0001$, $df = 43$), and interaction ($p < 0.0001$, $df = 86$) to all have a significant effect on soil NO_3^- concentration. Comparing each individually by Bonferroni post-test found a greater number of dates with significantly different soil NO_3^- concentrations between G and NM (7), and G and EM (6) than NM and EM (2), with the majority of differences occurring between March and July.

Figure 4. Mean (a) water filled pore space (WFPS) (%) and (b) soil temperature ($^{\circ}\text{C}$) \pm SE (n = 8) from November 2008 to November 2009 from three land uses: G (\bullet), NM (\blacksquare) and EM (\blacktriangle).

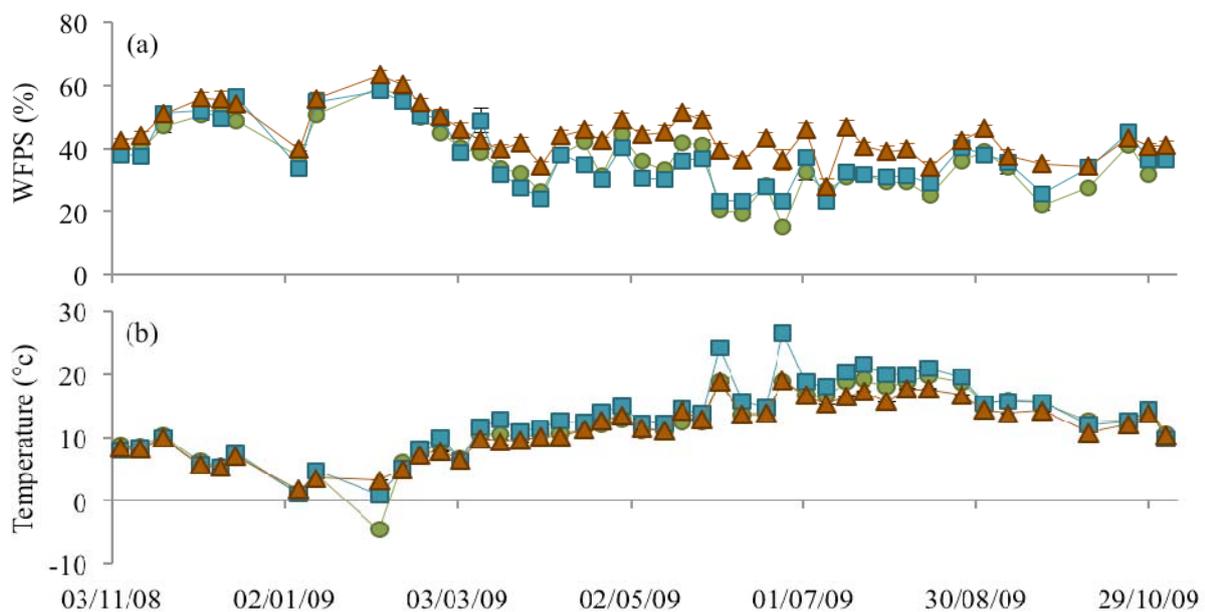
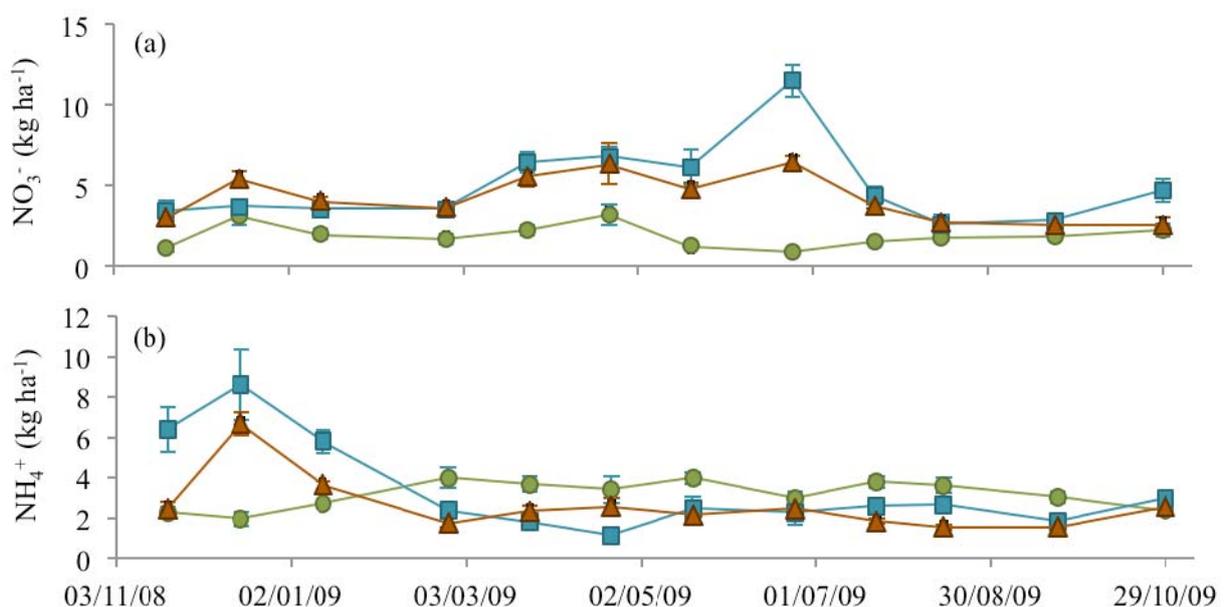


Figure 5. Mean (a) soil NO_3^- and (b) NH_4^+ ($\text{kg} \text{ ha}^{-1}$) \pm SE (n = 8) from three land uses: G (\bullet), NM (\blacksquare) and EM (\blacktriangle).



As with NO_3^- , Soil NH_4^+ concentration (0–10 cm) was measured monthly ($n = 8$) from all three treatments. NH_4^+ showed more variation between land uses than NO_3^- but had similar results when statistically analysed. The lowest mean soil NH_4^+ concentration ($1.1 \text{ kg}\cdot\text{ha}^{-1}$) was measured in NM on 22 April 2009, while the highest ($8.6 \text{ kg}\cdot\text{ha}^{-1}$), also in NM, was seen on 16 December 2008. Analysis by repeated measure two-way ANOVA showed land use ($p = 0.0037$, $df = 2$), time ($p < 0.0001$, $df = 43$), and interaction ($p < 0.0001$, $df = 86$) to all have a significant effect on soil NH_4^+ concentration. Again comparing each land use individually by Bonferroni post-test found a greater number of dates with significantly different soil NH_4^+ concentrations between G and NM (6), and G and EM (5) than NM and EM (2).

Further illustrated in Figure 5 is the difference in mean soil nitrate and ammonium concentrations between EM and G, and NM. A pattern can be seen whereby variation in both compounds exhibited contrary patterns throughout the year. While the difference in NO_3^- increased from spring through summer, the opposite was seen with NH_4^+ , which was greater during the winter months.

4. Discussion

In this experiment the difference in N_2O fluxes, and common factors that are known to affect them, were examined in three adjacent plots containing grassland (G), a newly planted *Miscanthus* crop (NM) and a long established *Miscanthus* crop (EM). A randomized block experimental layout was not possible due to the need to use a well-established *Miscanthus* plot. Hence a split-plot design with land use as the main treatment was used. All three plots share a very close geographical location, soil type, and land-use history. Recently a comparable study has been carried out using the same plots [22].

N_2O flux from all three land uses displayed large variation, with few noteworthy peaks measured throughout the year (Figure 2). Overall cumulative yearly fluxes were relatively low (216 ± 163 , 613 ± 294 , and $377 \pm 132 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ from G, NM, and EM respectively) and fell within the range commonly observed for unfertilised grasslands dominated by perennial ryegrass (*Lolium perenne*). In a nearby unfertilised grazed pasture, yearly fluxes of $1,000 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}$ have been recorded [28], while yearly N_2O fluxes of $219\text{--}511 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}$ have been observed in an unfertilised grassland in Scotland [29]. A recent study monitoring N_2O emissions from a 3–4 year old unfertilised *Miscanthus* crop over two years found mean annual emissions of $324 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$, again very similar to those found here [30]. Furthermore, the yearly cumulative N_2O emissions measured during this experiment compare favourably with those from other bioenergy crops. Hellebrand *et al.* [31] monitored N_2O emissions from a range of bioenergy crops. Yearly emissions from unfertilised plots in 2000 were; willow ($540 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}$), poplar ($530 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}$), hemp ($940 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}$), and rape ($1,110 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}$). Elsewhere, Jin *et al.* [32] have found yearly N_2O emissions ranging from $600\text{--}700 \text{ g}\cdot\text{N}\cdot\text{ha}^{-1}$ from unfertilised reed canary grass. Gauder *et al.* [33] have also found low N_2O emissions from unfertilised bioenergy crops, reporting N_2O emissions close to zero from both *Miscanthus* and willow. While recognising that factors other than crop type play an important role in soil N_2O production, it is clear that soil N_2O emissions from *Miscanthus* occupy a range commonly seen for unfertilised bioenergy crops.

Analyses by best fit multiple regression were inconsistent amongst land uses, with little more than a vague relationship observed. Adjusted R^2 squared values were poor ranging from 0.25 in NM to 0.02

and 0.06 in G and EM respectively. Of the 25% of N₂O fluxes accounted for from NM the interaction between soil NO₃⁻ concentration, WFPS, and soil temperature, and the interaction between soil NO₃⁻ concentration, soil NH₄⁺ concentration, WFPS, and soil temperature, were found to be highly significant. These analyses indicate that a large number of complex factors and interactions as opposed to any single variable are likely to influence the rate of N₂O flux throughout the year.

N₂O consumption was common, occurring in 36%, 40% and 42% of all measurements in NM, EM and G respectively. The similarity of uptake frequencies indicates a lack of any crop effect. The frequently cited link between N₂O consumption and low concentrations of soil inorganic N [34–36] is likely to have been an important factor in this case. N₂O consumption is thought to generally occur when N₂O is reduced to N₂ by denitrifiers during denitrification [37,38], although nitrifiers are also known to contribute to N₂O consumption via the nitrifier denitrification pathway [39–41]. It is unclear as to what was the main N₂O consumption pathway here. A best fit multiple regression analysis of N₂O versus WFPS, soil temperature, and nitrate and ammonium concentrations, on only occasions where N₂O uptake occurred yielded no significant results. Most of the peaks observed in N₂O emission and uptake occurred between 22 April 2009 and 22 July 2009. This period coincides with peak crop growth at the site, when competition for soil nitrogen between plants and soil microorganisms would be at its greatest. Similar results were found by Du *et al.* [42] who found an increase in N₂O flux variation during the growing season in grassland soils. The difference in WFPS between the sites was also found to increase during this time and may be linked. Dobbie and Smith [25] have reported WFPS to be the main driving factor behind N₂O flux during the growing season.

While land use was not found to significantly affect N₂O flux, a trend towards higher fluxes from the newly planted *Miscanthus* can be seen when looking at cumulative yearly fluxes (Figure 3). This difference may be due to soil disturbance and substrate incorporation caused by conventional tilling before *Miscanthus* was planted. Studies have found N₂O emissions to increase significantly following the disturbance of previously undisturbed soils. MacDonald *et al.* [43] found a significant increase in N₂O emissions in the year following the ploughing of a managed grassland, while Velthof *et al.* [44] have found that the renovation of a grassland increased N₂O emissions by a factor of 1.8–3.0. Furthermore, it has been reported that much of this effect can occur in a short period following tillage. Davies *et al.* [45] measured N₂O emissions of 1.5–3.7 kg·N·ha⁻¹ in the seven weeks proceeding ploughing. MacDonald *et al.* [43] have linked this with rapid rate of N mineralisation following soil disturbance. This experiment has not looked at these short-term effects. Work measuring the fluxes immediately following land use change to *Miscanthus* is currently being carried out in Ireland, and should be taken into account when considering the full N loss implications of land use change to *Miscanthus*.

As well as N₂O fluxes, soil bulk density, WFPS, temperature, nitrate and ammonium concentrations were measured. Bulk density was found to be lowest in G and highest in NM. This is due to the fact that NM was found to contain a high density of stones beneath the soil surface. These were moved towards the surface during tillage 11 months prior to these measurements. Bulk density was found to differ significantly between G and NM. This is unsurprising as G is long established and with no significant disturbance in its recent history, while NM was subject to considerable disturbance when tilled. Bulk density did not differ significantly between the other land uses. Bulk density has been used to calculate WFPS, and as such land use was found to have a significant effect on WFPS. WFPS was

found to be significantly different between G and EM, and NM and EM, on a substantially greater number of dates than between G and NM. Canopy cover is likely to have had a strong influence on these differences. A dense canopy was present at peak growth in EM compared to NM and G. A large leaf area will have affected soil water by increasing leaf evapotranspiration [46], as well as increasing the interception of radiant energy hence decreasing soil moisture losses by evaporation in EM [47]. The largest differences between treatments occur between the sampling dates 22 April 2009 and 22 July 2009, with WFPS being consistently higher in EM than G and NM. This is due to the emergence of crop cover as discussed above, but could also be affected by water use efficiency during this period of intense growth. C₄ plants are known to be capable of better water use efficiency than C₃ plants [48]. Land use was also found to affect soil temperature, with significant differences seen between all land uses (Figure 4b). This will have been affected by crop cover as discussed previously, but could also be linked with the differing bulk densities observed, as bulk density can directly affect soil temperature [49].

As none of the treatments were subject to additional nitrogen treatments soil nitrate and ammonium concentrations were found to be relatively low (0.9–11.5 kg·NO₃⁻·ha⁻¹ & 1.1–8.6 kg·NH₄⁺·ha⁻¹) when compared with conventionally fertilised arable sites. Both soil NO₃⁻ and NH₄⁺ concentrations were found to be significantly affected by land use. Significant differences occurred more frequently between G and NM, and G and EM, than NM and EM for both soil nitrate and ammonium concentrations. It is also apparent that temporal differences between NO₃⁻ and NH₄⁺ occur. NO₃⁻ increased in both *Miscanthus* treatments compared to the grassland throughout the growing season before decreasing again, whilst NH₄⁺ levels were greater in *Miscanthus* during the winter months before dropping below grassland concentrations during the growing season. These trends suggest differences in soil nitrogen cycling pathways. That these patterns were observed in both *Miscanthus* treatments when compared to the grassland suggests a crop effect. Foereid *et al.* [50] found a higher rate of soil N mineralisation in C₃ grass (*Lolium* spp) than *Miscanthus* during soil incubation experiments, while field studies have shown higher rates of soil N mineralisation in C₃ communities than C₄ during the growing season [51,52]. Slower rates of nitrogen mineralisation beneath C₄ species has been linked with an increase in the quantity and quality of plant litter, this in turn can lead to an increase in rates of microbial nitrogen immobilisation [53]. This could explain the large positive difference in soil NH₄⁺ between the grassland and *Miscanthus* treatments during the growing season. The difference in NO₃⁻ observed between the grassland and *Miscanthus* treatments during the growing season may indicate *Miscanthus* has a preference for root uptake of NH₄⁺. Suspension cultures of a similar species *Miscanthus x ogiformis* Honda “Giganteus” have been found to show a strong preference for ammonium as a source of nitrogen for growth [54].

5. Conclusions

Based on the results of this experiment, land-use change from grassland to *Miscanthus* will not have a negative impact on medium to long-term N₂O emissions. However, it is possible that there was a short-term increase in N₂O emissions immediately after establishment but prior to monitoring began. No significant difference was found in N₂O flux between the grassland and *Miscanthus* plots. Furthermore once established, *Miscanthus* can be a particularly low source of N₂O, with yearly N₂O

fluxes found in this experiment similar to those from unfertilised un-grazed grassland. Another important consideration is the use of nitrogen fertiliser. While this study examines a scenario involving no nitrogen addition, fertilisation will be a key factor in N₂O emissions from many land use change scenarios. *Miscanthus*' low fertiliser requirements [55,56] have been found to lead to reduced N₂O emissions when compared to other agricultural crops [33,57].

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Conflicts of Interest

The authors declare no conflict of interest.

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